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CONGENITAL TRANSMISSION OF IMMUNITY TO VARIOLA IN WHITE MICE

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Abstract

White mice immunized with variola virus transmit the acquired protection congenitally ("deplacental and trophogenic") to their young. Passive immunity of the litters can be tested by infection with variola virus. The system appears suitable for qualitative and quantitative testing of pox vaccines and of antigens from the variola-vaccinia group.

Introduction

White mice are able to transmit actively acquired immunity to their young. The mechanism of transmission was investigated in detail through infection with foot-and-mouth disease in white mice (Ref. 2, 5, 12). It was further investigated whether the transmission of the maternal antibodies to the infant mice is suitable as a method of testing the antigenic properties of hoof-and-mouth disease inocula (Ref. 3, 4). Since baby mice are highly sensitive to foot-and-mouth disease infection and their passive immunity acquired from the inoculated mother can be easily tested, such a method of testing seemed suitable. Similar conditions are also given in the variola infection of white mice. Variola virus is highly virulent for infant mice whereas older animals resist infection.

This report investigates the qualitative and quantitative conditions in the transmission of actively acquired maternal immunity against variola to infant mice. The investigation is simultaneously intended to clarify whether a simple method can be developed from this model with which the cross-immunizing properties of variola and vaccinia virus strains can be tested through infection in small experimental animals.

Materials and Methodology

All tests were carried out with the variola strain Bombay 1958. Since its isolation (Ref. 6), the strain has undergone numerous passages in chicken egg, on various tissue cultures and in infant mice. Tissue-culture virus from 50 to 52 passages in muscle cells of calf embryos was preferred because of its somewhat weaker pathogenicity to baby mice for first infections and booster inoculation. For the test of immunity to infection, we utilized exclusively the virus after 10 to 20 intraperitoneal passages in infant mice. The virus was obtained from acitic exsudate and from triturated kidney, lungs and spleen and was stored at -65° C. The experimental animals were from our own breed of mice free of ectromelia (Breed II of the Federal Research Institute for Virus Diseases of Animals in Tubingen). The test groups were selected from litters of several families so as to furnish average populations. All solutions of virus strain were titrated on the chorio-allantoic membrance (CAM) of the incubated chicken egg and the results evaluated by the pox-counting method. In addition, we determined, prior to utilizing any virus sample, LD_{50} for 2-3 day infant mice. LD_{50} was calculated from Reed and Muench and the infective and virus dosages adjusted from the result. The titration data are referenced to 0.1 ml. The sera required for serological reaction were always cumulated sera of several and/or many animals, especially for infant mice. We utilized primarily the not otherwise needed males of the litters. Reaction to hemagglutination inhibition was made by customary methods. The neutralizing antibodies were titrated by the serum-neutralization test (serum dilution of the second power to constant virus amount) against a culture virus forming 50 plaque units (PBE). The mixtures were inoculated to tissue culture from muscle cells of calf embryos. They were grown in Leyton tubes and contained pure bovine amniotic fluid as culture medium. Reduction of the plaque number by 50% and more in relation to the control was considered as positive value.

Findings

We investigated in preliminary tests whether infant white mice form better immunity to variola infection through intraperitoneal or subcutaneous application. Immunity is preserved

to adult age and can be improved and prolonged through booster inoculation. As an expression of the humoral immunity, it is possible to demonstrate significant values of virus neutralizing and hemagglutination-inhibiting antibodies -- the latter only by animals infected intraperitoneally -- in the blood of the animals. For an understanding of variola infection in infant mice, it is important to know that the mode of infection plays an essential role. The principles can be summarized as follows (Ref. 1, 7-11).

After intraperitoneal infection, the result is a function of infective dose and age of the animals. For 1-day mice, 1-10 PBE correspond to LD_{50}; however, 3-day mice require a lethal dose of 1,000 PBE. Amounts of virus up to 10^{-3} is supported by the animals after slight sickness. Infant mice 5 days old support as much as 10^5 PBE. After 6 days, there occurs a definite drop of sensitivity and even 10^7 PBE are only moderately lethal (as a function of litter) in 6-day animals. After this infective dose, 9-day animals survive without showing visible symptoms. Increasing infective dose shortens the period of incubation in infant mice 1-5 days old and the duration of the sickness until death (2/5 and/or 3/12 days). High doses are occasionally fatal after 2 days in mice 1-3 days old. Virus concentrations of as much as 10^9/ml can be demonstrated in the internal organs and the large amount of acitic fluid of the diseased and dead animals. Recovered animals may carry the virus for 1 month. Intracerebral infection is about 10 times more effective than intraperitoneal infection and is characterized by pronounced encephalitic symptoms. Lethality closely approaches the threshold of infection even in animals several days old. The virus propagates very strongly also in the brain but the extent in the internal organs does not reach that occurring under intraperitoneal infection. Subcutaneous infection, even with high doses does not cause infant mice to fall sick. However, the virus propagates for a short time in the internal organs, especially in the lung, and reaches concentrations above 10^5/ml. The same holds for intracutaneous infection. Percutaneous inoculation does not take in infant mice.

On the basis of these circumstances, we subsequently tested the extent and manner of transmission to their young of immunity to variola produced in gravid mice. For this purpose we formed the following groups of differently immunized mice:

Group A-ip: infant mice infected intraperitoneal once with 10^3 PBE of variola virus.

Group A-sc: infant mice subcutaneously infected once with 10^6 PBE of variola virus.

Group B-ip: infected like Group A-ip, with booster inoculation at intervals of 1 week.

Group B-sc: infected like Group A-sc and booster inoculation at intervals of 1 week.

Group C: pregnant mice (4th, 9th, 15th, and 17th day of pregnancy inoculated intraperitoneal with 10^6 PBE each of variola virus.

We also inoculated intraperitoneal pregnant animals once at different times (cf. Table 1).

The litters of all females of these groups were infected intraperitoneal with variola virus 3 days after birth and compared to controls from non-immunized mothers. The result is grouped in Table 1.

Table 1 - Findings from Infection with 10-1,000 LD_{50} of Infant Mice 3 Days Old from Differently Infected and Immunized Mothers

Gruppe / Immunisierung der Muttermäuse	Babymäuse: Testtiere tot/insgesamt	Babymäuse: Kontrollen tot/insgesamt
Gruppe A-i.p.	0/52	32/32
Gruppe A-s.c.	0/15	10/10
Gruppe B-i.p.	0/76	25/25
Gruppe B-s.c.	0/87	18/18
Gruppe C	0/119	
einmalig am 7. Trächtigkeitstag	0/42	
einmalig am 10. Trächtigkeitstag	0/66	
einmalig am 12. Trächtigkeitstag	3/32	29/29
einmalig am 15. Trächtigkeitstag	4/85	
einmalig am 17. Trächtigkeitstag	58/62	
einmalig am 18./19. Trächtigkeitstag	30/30	

a = mother mice immunized once on x-th day of pregnancy; b = infant mice; c = test animals - died/total; d = controls - died/total

The table indicates that all infant mice obtained sufficient passive protection from their actively immunized mothers in order to support 1,000 LD_{50} of variola virus. The mothers must have received at least once in their life a strong dose of variola virus not later than the 10th day of pregnancy. Pregnant mice inoculated 4 days or less before having their young no longer develop sufficient immunity to protect their young as compared with other infection-tested litters.

A part of the litters of the Groups A and B was not infected but all blood withdrawn in intervals after birth. In all sera, only neutralizing antibodies could be demonstrated although high values of hemagglutination-inhibiting antibodies could be demonstrated in the blood of the mothers of the groups inoculated intraperitoneal. The neutralizing antibodies attained an appreciable density but persisted only until 3 weeks after birth.

The 2nd litters of mother mice of Group B -- not further treated with variola virus after the first litter -- also all supported infection with 1,000 LD_{50} of variola virus. In the non-infected siblings, serologically demonstrable amounts of neutralizing antibodies were found but these persisted only for a few days after birth. Only 50% of the 3rd litters resisted test infection.

Table 2. Mortality Rate (Died/Total) of Infant Mice Infected Intraperitoneal 3 Days After Birth from Mothers Immunized Once and Repeatedly Grouped by Infective Dose (n.d. = not made)

[a] Gruppen der Muttermäuse	A-i.p.	A-s.c.	B-i.p.	B-s.c.	[b] Kontrollen
10 LD_{50}	0/21	n. d.	0/20	0/18	20/20
100 LD_{50}	0/19	0/7	0/22	0/27	28/28
1000 LD_{50}	0/42	0/8	0/34	0/42	31/31
10000 LD_{50}	n. d.	n. d.	0/23	n. d.	8/8

a = groups of mother mice; b = controls.

Table 2 groups the findings of test infection in infant mice of the 4 groups in Table 1 grouped by LD_{50} dose. In all groups, the threshold of "breakthrough" could not be attained with the test-virus suspensions utilized. The lethal dose for intraperitoneal infection of mice 3 days old is 1,000 PBE. Accordingly, test-virus suspensions of 10^6 PBE per 0.1 ml are required to attain 1,000 LD_{50} for 1 dose. A test virus with a titer of 10^4 LD_{50} (10^7 PBE/0.1 ml) was available only in a partial test.

The strongly passive protection of the litters of mother mice immunized to variola is composed of antibodies transmitted diplacental and trophogenic. A further test showed the manner in which these 2 types of transmission of immunity participate in the passive protection of the infant mice. The litters of differently immunized groups were removed from their mothers immediately after birth and given to non-treated females for

nursing. The "pure" litters of the control mothers were given to the treated mothers. All infant mice of this test were infected with 1,000 LD_{50} of variola virus 3 days after birth. The results are shown in Table 3. Differences in the degree of immunity obtained in the different groups and transmitted to the litters were shown here also.

Table 3. Mortality Rate (Died/Total) of Infant Mice Infected Intraperitoneal with 1,000 LD_{50} 3 Days After Birth from Immunized Mother Mice when Nursed by Non-treated Mothers (+ = death retarded)

a Babymause / Mutterliere	b „reine"	A-i.p.	A-s.c.	B-i.p.	B-s.c.	C
c „reine" Ammen	107/107	8/32	10/31	7/33	0/60	20+/54
Group Gruppo A-i.p.	18/20	0/82	—	—	—	—
Gruppo A-s.c.	43/50	—	0/15	—	—	—
Gruppo B-i.p.	0/41	—	—	0/70	—	—
Gruppo B-s.c.	0/03	—	—	—	0/87	—
Gruppo C	0/54	—	—	—	—	0/110

a = mother mice/infant mice; b = "pure"; c = "pure" nursing mothers

Mother animals receiving variola virus only once as infants (A-ip and A-sc) were able to protect through their milk less than 50% of the "pure" infant mice within the first days of life. Diplacental protection of infant mice of the Group A-sc was just sufficient so that less than 50% survived the test infection.

Discussion

White mice react to infection and/or inoculation with variola virus by forming a stable immunity. The latter can be increased and prolonged by booster inoculation. The protection of mother mice is transmitted diplacental and trophogenic to the litters. Such infant mice have shortly after birth a strong passive immunity which can be tested by infection with variola virus.

The greater part of the protection obtained through the mother appears to consist of virus-neutralizing antibodies whereas hemagglutination-inhibiting antibodies are not transmitted. This makes it possible to qualitatively and

quantitatively test immunity to pox obtained in mice through infection test on litters. The satisfactory passive immunity of infant mice offers a sufficiently wide range for graduation of the test infection. Further possibilities of variation exist in regard to path, instant in time, and dose of immunization of the mother mice as well as in the point in time of the test infection after birth of the infant mice. It should be possible on this basis to disclose qualitative differences under uniform methodology through comparison of antigen-effective preparations and various strains.

Since vaccinia virus satisfactorily immunizes mice (protective inoculation against ectromelia), the method might be initially developed into a very significant test for comparative qualitative testing of virulent and inactivated pox inocula. An additional advantage is the possibility of testing against variola virus. The method further seems appropriate for testing cross immunity within the pox group and for investigating the immunizing properties of different vaccinia-virus strains to the virus of variola.

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